Attorney Docket No: 18390,2032 (Serial No.: 09/886,899)
Inventor: Lovell-Badge, *et al.*
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REMARKS

Claims 1-8, 16 and 17 are pending.

The invention relates to a method for isolating a pluripotent cell which is at least partially committed to a given developmental pathway, that includes the step of sorting a population of pluripotent cells according to Sox gene expression.

Double Patenting

The Examiner states that claims 1-8, 16 and 17 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-8 of copending Application No. 09/464,146.

In response to this rejection, Applicants will submit a terminal disclaimer to disclaim any portion of a patent issuing from the present application which would extend beyond the term of a patent issuing from the 09/464,146 application, upon notification of allowable claims in the present application.

Rejection of Claims 1-8, 16 and 17 under 35 U.S.C. §112, First Paragraph

Claims 1-8, 16 and 17 remain rejected under 35 U.S.C. §112, first paragraph, for alleged lack of enablement.

Applicants respectfully traverse this rejection.

Breadth of the Claims

Any pluirpotent cell, organism or Sox gene

The Examiner again asserts that "the claims broadly encompass the use of any pluripotent cell derived from any organism whatsoever and any Sox gene. Further, the claims are broadly drawn to the use of sorted cells."

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Claim 1 and dependent claims 2-8, 16 and 17 have been amended to be limited to any one of the Sox genes "selected from the group consisting of: *Sox21* (GenBank Accession No. AF107044); *Sox14* (GenBank Accession No. 107043); *Sox13* (GenBank Accession No. AB104474); *Sox10* (GenBank Accession No. AJ001183); *Sox22* (GenBank Accession No. U35612); *Sox18* (GenBank Accession No. L35032); *Sox11* (GenBank Accession No. U23752); *Sox1* (GenBank Accession No. Y13436); *Sox2* (GenBank Accession No. Z31560 and U12532); *Sox3* (GenBank Accession No. X94125); *Sox4* (GenBank Accession No. X70683); *Sox5* (GenBank Accession No. S83306); *Sox6* (GenBank Accession No. U32614); *Sox7* (GenBank Accession No. AI15903/P40646); *Sox9* (GenBank Accession No. S74504/5/6); *Sox12* (GenBank Accession No. U70442); *Sox13* (GenBank Accession No. AB006329); *Sox15* (GenBank Accession No. AB104474); *Sox16* (GenBank Accession No. L29084); *Sox17* (GenBank Accession No. D49473); *Sox19* (GenBank Accession No. X98368); *Sox22* (GenBank Accession No. U35612)."

The claims no longer encompass any Sox gene but are limited to the 22 Sox genes recited in the claim. The claimed method is limited to the use of only those cell types and those organisms that express a Sox gene with the accession number recited in claim 1. Applicants submit that in view of all of the above, amended claim 1 and dependent claims 2-8, 16 and 17 are properly enabled.

Sorted Cells

The Examiner continues to assert at page 6 that "[t]he claims are also drawn to a broad use of sorted cells, after detection by some means. Since only two modes are known for detection followed by FACS sorting in the prior art, which rely on either antibody or nucleic acids for the detection, and no additional disclosure is provided in the specification on methods of such sorting, the claims are limited to the prior art methods, which all teach the use of dead cells."

Applicants respectfully disagree.

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Claim 8 has been amended to claim "the method of claim 1, wherein said *Sox* gene expression is detected by staining for β-galactosidase activity."

The specification teaches in Example 6, and in particular at page 53, line 22 through page 54, line 5, a method of isolating viable cells by sorting the cells according to Sox gene expression. It is stated at page 53, line 22 through page 54, line 5, "[t]o attempt to isolate the neural progenitor pool, ES cells are used in which the bifunctional selection marker/reporter gene βgeo has been integrated into the *Sox2* gene by homologous recombination. When induced to differentiate as described above, approximately 50% of these cells stain for β-galactosidase activity, consistent with the proportion of cells that express *Sox2* protein. Therefore, application of G418 to the differentiating cultures should eliminate *Sox2*-negative non-neural cells. G418 (200 g/ml) is added after retinoic-acid induction, either during embryoid body culture or upon plating. In both conditions appreciable cell killing is evident. **Crucially, however, large numbers of cells survive that exhibit the small, ovoid morphology typical of neuroepithelial cells.** Over 90% of these cells show prominent β-galactosidase staining. Expression of *Sox1* and *Sox2* proteins is confirmed by immunostaining. Consistent with a neuroepithelial identity, the cells also express nestin." (Emphasis added) Applicants submit that it was known in the art as of the filing date of the instant application that neural stem cells specifically express nestin (Lendahl et al., 1990, Cell 60:585-595; Dahlstrand et al., 1992, J Cell Sci., 103 (Pt 2):589).

In view of the above, Applicants submit that the specification clearly teaches a method of sorting cells according to Sox gene expression wherein a viable population of cells are isolated.

Quantity of Experimentation

The Examiner continues to assert at page 6 that "[t]he quantity of experimentation in this area is large since there is significant variability in the function and activity of each of the Sox genes in each of the cell types and different sources...In order to use any given Sox gene, abundant and inventive experimentation would be necessary in order to determine the biological and molecular roles of the molecule."

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Applicants respectfully disagree.

The invention as claimed in claim 1 relates to the use of Sox gene expression as a marker for a pluripotent cell which is at least partially committed to a given developmental pathway. The method of the claim requires expression of a Sox gene selected from the group of 22 Sox genes recited in amended claim 1. Claim 1 does not require knowledge of the "biological and molecular roles of the molecule [Sox gene]", as asserted by the Examiner.

Unpredictability

The Examiner states at page 7 that "the art teaches that it is entirely unpredictable what function Sox genes have in cells." The Examiner also states that "[g]iven the unpredictability found in the known Sox genes, it is even more unpredictable what effects as yet unidentified Sox genes in the tens of thousands of different possible cells would have."

Applicants respectfully submit that the invention as claimed in amended claim 1 relates to the use of Sox gene expression as a marker for a pluripotent cell which is at least partially committed to a given developmental pathway. The method of amended claim 1 requires expression of a Sox gene selected from the group consisting of the 22 Sox genes recited in the claim. Claim 1 does not require knowledge of the "function Sox genes have in cells", as asserted by the Examiner. Further, amended claim 1 is limited to known Sox genes therefore rendering the issue of unpredictability of as yet unidentified Sox genes moot.

The Examiner also states that "[a] separate area of unpredictability concerns the isolation and sorting of the Sox expressing cells...it is unpredictable how a 'pluripotent' cell can be isolated using a intracellular marker without killing the cell and thereby rendering the cell not 'pluripotent'."

As stated above, the specification teaches in Example 6, and in particular at page 53, line 22 through page 54, line 5, a method of isolating viable cells by sorting the cells according to Sox gene expression. Example 6 teaches further at page 54, lines 6-25,

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"Accordingly, neural cell types may be isolated by expression of a marker associated with *Sox2*, starting with a population of totipotent cells which has been induced to differentiate *inter alia* into a neural pathway.

In order to determine whether the *Sox2*-selected population have proliferative capacity, βFGF is added to plated cultures. This results in a major stimulation of cell division. The expanded cells predominantly retain undifferentiated neural morphology and show strong X-gal staining indicative of *Sox2* expression. Such cultures can be amplified and serially passaged for at least three weeks, which is significantly longer than the proliferative phase of neurogenesis in the mouse embryo.

In the absence of mitogen, *Sox2*-selected precursor cells begin to extend neuritic processes within 48 hours and by 96 hours form a network of neuron-like cells. The pan-neuronal markers neurofilament light chain, microtubule-associated proteins, MAP2 and tau, and β-tubulin III are detectable from 48 hours onwards, coincident with down-regulation of *Sox2* expression. By 96 hours, over 90% of cells express neuronal markers, including neurofilament heavy chain and synapsin I. Cells of non-neuronal morphology are rarely apparent, with the exception of the occasional GFAP-positive astrocyte. Astrocyte numbers increase if serum of FGF is added to the cultures. Maturation of the neuronal cells, evidenced by production of gamma-aminobutyric acid (GABA) and glutamate neurotransmitters, and further elongation of neurites with dendritic sprouting is achieved on transfer to Neurobasal medium supplemented with B27 and horse serum."

As recited above, a pluripotent cell, as defined in the instant specification, includes "neural stem cells".

In view of the above, Applicants submit that the specification teaches a method of sorting cells according to Sox gene expression wherein a viable population of pluripotent cells are isolated.

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Working Examples

In response to the Examiner's statement that "[t]he specification has no working examples of isolation of pluripotent cells by detection of Sox gene expression followed by sorting of the cells", Applicants assert that Example 6 teaches a method of sorting cells according to Sox gene expression wherein a viable population of pluripotent cells are isolated.

Guidance in the Specification

The Examiner states that "[t]he specification...does not teach the sequence of Sox genes from even a representative fraction of all of the different organisms which are included in the scope of the claim, nor are a representative number of precursor cell types given."

The Examiner also states, "[w]hile the specification does have a table...which discusses 24 Sox genes and lists a few different species in which these genes are found, in only a very few of these situations is any biological role known."

The Examiner also states, "the specification provides no disclosure of how to isolate living pluripotent cells".

Claim 1 has been amended to add the limitation of "detecting expression of a Sox gene selected from the group consisting of: selected from the group consisting of: *Sox21* (GenBank Accession No. AF107044); *Sox14* (GenBank Accession No. 107043); *Sox13* (GenBank Accession No. AB104474); *Sox10* (GenBank Accession No. AJ001183); *Sox22* (GenBank Accession No. U35612); *Sox18* (GenBank Accession No. L35032); *Sox11* (GenBank Accession No. U23752); *Sox1* (GenBank Accession No. Y13436); *Sox2* (GenBank Accession No. Z31560 and U12532); *Sox3* (GenBank Accession No. X94125); *Sox4* (GenBank Accession No. X70683); *Sox5* (GenBank Accession No. S83306); *Sox6* (GenBank Accession No. U32614); *Sox7* (GenBank Accession No. AI15903/P40646); *Sox9* (GenBank Accession No. S74504/5/6); *Sox12* (GenBank Accession No. U70442); *Sox13* (GenBank Accession No. AB006329); *Sox15* (GenBank Accession No. AB104474); *Sox16* (GenBank Accession No. L29084); *Sox17* (GenBank Accession No. D49473); *Sox19* (GenBank Accession No. X98368); *Sox22* (GenBank

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Accession No. U35612). Amended claim 1 is limited, therefore, to Sox genes having a known sequence.

As discussed above, amended claim 1 does not require knowledge of the biological role of the Sox genes of claim 1.

Further, as stated above, Example 6 teaches a method of sorting cells according to Sox gene expression wherein a viable population of pluripotent cells are isolated.

In view of all of the above, Applicants submit that claims 1-8 and 16 and 17 are properly enabled and respectfully request reconsideration and withdrawal of the 35 U.S.C. § 112, first paragraph rejection of claims 1-8, 16 and 17.

Rejection of Claims 1-8 and 16 under 35 U.S.C. §112, First Paragraph

Claims 1-8, 16 and 17 remain rejected under 35 U.S.C. §112, first paragraph, for allegedly failing to meet the written description requirement.

Applicants respectfully traverse this rejection.

The Examiner states that "[a]ll of the current claims encompass a genus of nucleic acids which are different from those disclosed in the specification. The genus includes variants for which no written description is provided in the specification since the genus is open to any allelic variant of the current Sox genes as well as any new Sox gene discovered in any species whatsoever. Thus, applicant has express possession of only twenty or so particular Sox genes in a genus which comprises hundreds of millions of different possibilities."

The Examiner also asserts that "the definition of Sox genes in the specification is entirely functional...without any structural limitations...specific knowledge of what a "SOX" protein or nucleic acid consists of is absent...[I]n the application at the time of filing, there is no record or description which would expressly demonstrate conception of any nucleic acids other than those expressly disclosed which comprise Sox genes or proteins."

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Claim 1 has been amended to add the limitation of "detecting expression of a Sox gene selected from the group consisting of "*Sox21* (GenBank Accession No. AF107044); *Sox14* (GenBank Accession No. 107043); *Sox13* (GenBank Accession No. AB104474); *Sox10* (GenBank Accession No. AJ001183); *Sox22* (GenBank Accession No. U35612); *Sox18* (GenBank Accession No. L35032); *Sox11* (GenBank Accession No. U23752); *Sox1* (GenBank Accession No. Y13436); *Sox2* (GenBank Accession No. Z31560 and U12532); *Sox3* (GenBank Accession No. X94125); *Sox4* (GenBank Accession No. X70683); *Sox5* (GenBank Accession No. S83306); *Sox6* (GenBank Accession No. U32614); *Sox7* (GenBank Accession No. AI15903/P40646); *Sox9* (GenBank Accession No. S74504/5/6); *Sox12* (GenBank Accession No. U70442); *Sox13* (GenBank Accession No. AB006329); *Sox15* (GenBank Accession No. AB104474); *Sox16* (GenBank Accession No. L29084); *Sox17* (GenBank Accession No. D49473); *Sox19* (GenBank Accession No. X98368); *Sox22* (GenBank Accession No. U35612)." Claim 1 therefore encompasses only the 22 Sox genes recited in the claim and does not encompass a genus of nucleic acids which are different from those disclosed in the specification.

In view of all of the above, Applicants submit that claims 1-8, 16 and 17 meet the legal requirement for written description, and respectfully request reconsideration and withdrawal of the 35 U.S.C. § 112, first paragraph rejection of claims 1-8, 16 and 17.
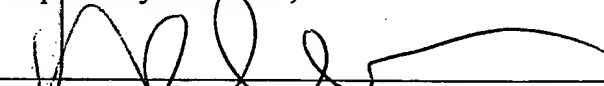
CONCLUSION

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner.

Respectfully submitted,

Date: _January 21, 2004_

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